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Role of cytokines and testosterone in regulating lean body mass and resting energy expenditure in HIV-infected men

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¹Department of Community Health and Department of Medicine and ²Nutrition, Exercise Physiology, and Sarcopenia Laboratory, Jean Mayer US Department of Agriculture Human Nutrition Research Center, Tufts University, Boston 02111; ³Division of Endocrinology, Massachusetts General Hospital, Boston 02114; and ⁴Departments of Epidemiology and Biostatistics, Harvard School of Public Health, Boston, Massachusetts 02115

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Roubenoff, Ronenn, Steven Grinspoon, Paul R. Skolnik, Eric Tchetsgen, Leslie Abad, Donna Spiegelman, Tamsin Knox, and Sherwood Gorbach. Role of cytokines and testosterone in regulating lean body mass and resting energy expenditure in HIV-infected men. *Am J Physiol Endocrinol Metab* 283: E138–E145, 2002. First published March 27, 2002; 10.1152/ajpendo.00426.2001.—Although catastrophic weight loss is no longer common in HIV-infected men, we hypothesized that a more gradual process of cachexia [loss of lean body mass (LBM) without severe weight loss, often accompanied by elevated resting energy expenditure (REE)] is still common and is driven by excessive production of the catabolic cytokines tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β). We performed a longitudinal analysis of an ongoing cohort study of nutritional status in 172 men with HIV infection. LBM loss of >1 kg occurred in 35% of the cohort, and LBM loss of >5% occurred in 12.2% over 8 mo of observation, but classical wasting (loss of \approx 10% of weight) was rare (2%). Both TNF- α (-150 g LBM \cdot ng $^{-1}\cdot$ ml $^{-1}$, $P < 0.02$) and IL-1 β production (-130 g LBM \cdot ng $^{-1}\cdot$ ml $^{-1}$, $P < 0.01$) by peripheral blood mononuclear cells predicted loss of LBM. A rise in REE of >200 kcal/day was found in 17.7% of the subjects regardless of weight change. IL-1 β (+9 kcal/day per ng/ml, $P < 0.002$) and TNF- α (+10 kcal/day per ng/ml, $P < 0.02$) production predicted Δ REE. Serum free testosterone was inversely associated with TNF- α production and was not an independent predictor of either Δ LBM or Δ REE after adjustment for cytokine production. Even though weight loss was rare in this cohort of patients treated with highly active antiretroviral therapy, loss of LBM was common and was driven by catabolic cytokines and not by inadequate dietary intake or hypogonadism.

WITH THE ADVENT of highly active antiretroviral therapy (HAART), the incidence of most of the complications associated with HIV infection has declined in the developed world (17). However, the metabolic derangements associated with HIV infection have not been eliminated: wasting continues to occur even with HAART (17), and lipodystrophy, or fat redistribution syndrome, has been recognized as a new complication

of the disease and possibly of HAART treatment (3). Even though severe weight loss (wasting syndrome) appears to be less common today, erosion of lean body mass (LBM), primarily from skeletal muscle, is still evident in many patients with HIV infection when body composition is measured (8, 30). Because muscle mass is the predominant determinant of strength, loss of LBM can lead to loss of functional capacity and independence, and the resulting reduced protein stores diminish the ability to withstand an acute infection (12, 22).

We have previously demonstrated that such loss of LBM can occur even without loss of weight and have suggested that the term cachexia be used to distinguish this condition from the more severe weight loss seen in wasting (defined by the Centers for Disease Control as loss of 10% or more of baseline weight) (4, 12, 22). For example, in rheumatoid arthritis, excess production of the catabolic cytokines interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) by peripheral blood mononuclear cells (PBMC) drives cachexia and increased resting energy expenditure (REE) and protein catabolism but without overt weight loss (21, 24). Because rheumatoid arthritis is a systemic illness, as is HIV infection, we sought to determine whether PBMC production of catabolic cytokines is also an important determinant of cachexia in HIV-infected adults.

In AIDS wasting, Grinspoon and colleagues (10, 11) have demonstrated that low serum testosterone is an important determinant of LBM loss. However, it is not clear whether either testosterone or the catabolic cytokines are important factors in regulating weight, body composition, or metabolic rate in HIV-infected adults in the absence of overt wasting. We hypothesized that cachexia is a persistent problem in HIV-infected men despite treatment with HAART, and that, like rheumatoid cachexia, HIV-associated cachexia is driven by catabolic cytokine production. We examined the rela-

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tionship between the determinants cytokine production and serum testosterone concentration and change in LBM and REE in male participants in the Tufts Nutrition for Healthy Living Study, an ongoing longitudinal study of the nutritional status of HIV-infected adults.

PATIENTS AND METHODS

Study design. The Tufts Nutrition for Healthy Living Study began in 1993 and included 695 subjects as of May 2000. Subjects are contacted by telephone every month and are examined and fill out detailed questionnaires about their health. Every 6 mo, their body composition is measured by bioelectrical impedance (BIA) and their REE by indirect calorimetry. Between May 1995 and August 1998, a subgroup of 172 men who had adequate venous access to allow additional blood drawing donated peripheral blood mononuclear cells for assessment of cytokine production. Sufficient serum was available for measurement of free testosterone in 166 cases.

Body composition. Body composition was measured at each visit by single-frequency, whole body BIA using an RJL 103 analyzer (RJL, Mt. Clemens, MI). Electrodes were placed in standard positions on the wrist and ankle. LBM was calculated using sex-specific equations validated against dual-energy X-ray absorptiometry (DEXA) in this study population (14).

Indirect calorimetry. REE was measured by indirect calorimetry using a SensorMedics V2900 calorimeter (SensorMedics, Yorba Linda, CA). Measurements were made after a minimum of 4 h of fasting to eliminate the thermic effect of the last meal on REE. Subjects lay quietly in a semi-darkened, thermoneutral room for 20 min and then had their REE measured for an additional 15 min.

Cytokine measurements. PBMC were isolated from 20 ml of anticoagulated blood as previously described (24). PBMC were obtained from whole blood by Ficoll-Hypaque centrifugation, as previously described, and washed three times in sterile, pyrogen-free saline (9, 26). Cells were suspended at $5 \times 10^6/\text{ml}$ in RPMI (Sigma Chemical, St. Louis, MO) that had been subjected to ultrafiltration (6) to remove cytokine-inducing substances. Ultrafiltered RPMI was supplemented with 100 $\mu\text{g}/\text{ml}$ streptomycin, 100 U/ml penicillin (Sigma), and 1% L-glutamine and contained 2% autologous heat-inactivated serum. No additional stimulation was given to the cells. Cells were cultured in 96-well flat-bottom plates; each well contained 0.125 ml of cells plus 0.125 ml of RPMI. After 22 h at 37°C in 5% CO₂, the plates were frozen at -80°C. Plates were thawed and frozen three times to lyse the cells.

Measurement of total IL-1 β and TNF- α synthesis was carried out in duplicate by specific, non-cross-reacting enzyme-linked immunosorbent assays (ELISA, R&D Systems, Minneapolis, MN), as described previously (9, 29). Cell lysates were assayed undiluted. The interassay coefficient of variation (CV) for samples was <10%, and the intra-assay variability was <5% for both cytokines.

Free testosterone measurement. Serum free testosterone was measured by radioimmunoassay (Diagnostic Products, Los Angeles, CA). The intra-assay CV was 3.2–4.3%.

Other measures. Dietary intake was measured from 3-day food records analyzed using the Minnesota database (NDS-R, version 4.0, University of Minnesota Division of Epidemiology). Physical activity was estimated using the Physical Activity Recall Scale developed by Sallis et al. (25). CD4 counts were measured by flow cytometry in the New England Medical Center Clinical Immunology Laboratory. Plasma HIV

RNA was measured in a research retrovirology laboratory using a Roche Amplicor Monitor reverse transcriptase polymerase chain reaction assay according to the manufacturer's instructions (Roche Molecular Systems, Somerville, NJ). Blood samples were collected in ACD tubes, and plasma was separated within 3 h, frozen at -70°C, and thawed just before assay.

Statistical analysis. Descriptive statistics were calculated to inspect the distribution of all variables of interest in the analysis. These variables were chosen on the basis of a literature review of variables related physiologically to the outcomes of interest. Because most variables were distributed normally, means \pm SD are shown; for skewed variables, the median and interquartile range are shown. Intervals with a change in REE of >500 kcal or change in LBM of >5 kg were considered outliers ($n = 13$) and excluded from the analysis, as they are not physiologically likely. Repeated measurements were collected at standardized 6-mo study visits with the use of identical protocols by trained study personnel. Therefore, participants generally contributed multiple observations to our data set. The Statistical Analysis System (SAS, Cary, NC) software was used for all analyses. The SAS procedure MIXED was employed to fit all reported regression models.

Absolute changes in weight, LBM, and REE over a maximum time interval of 8 mo were modeled using repeated-measures regression methods (32). These models were employed to assess the presence of crude and multivariate-adjusted prospective associations between measured levels of cytokines and testosterone at the start of an interval, potential confounders measured at the start of the interval, and changes in weight, LBM, and REE over the interval. Empirical estimates of the variance of parameters were used with an unstructured covariance matrix, where the variance of the dependent variables at each time point measured, and all possible covariances between them, are allowed independent estimates.

Restricted cubic spline regression models were used to test significant nonlinearity in the independent association between the three dependent variables of interest and the main determinants (cytokines and testosterone) (7). Restricted cubic splines are a method for flexibly fitting arbitrary (i. e., not determined a priori) nonlinear regression relationships by joining a series of cubic polynomials at prespecified knot points, with smoothness constraints at these knots and with the tails fit linearly (7). These models do not assume a linear relationship between predictor and outcome, but rather fit any nonlinear behavior arbitrarily, and provide a test for nonlinearity by comparing the fit of the nonlinear model to the fit of the best linear one. The likelihood ratio test was employed to assess nonlinearity in the flexible regression models. Assumptions of linearity were carefully checked through the restricted cubic spline regression models described above. Graphs of the arbitrary regression relationships were inspected for visual evidence of nonlinearity, and statistical tests for nonlinearity were performed as described above. Independence between repeated measures on the same subjects was never assumed in this analysis. Rather, repeated measures were treated as correlated, by an arbitrary correlation matrix as described above.

Data on cytokines, testosterone, and changes in LBM, weight, and REE were available for different subsets of the available person-intervals. For the change in LBM analysis, there were 166 and 147 person-intervals with cytokine and testosterone data, respectively, and 73 with both cytokine and testosterone. For the change in REE analysis, there were 132, 109, and 58 person-intervals available with cytokine,

testosterone, and both, respectively. The missing indicator method (16) was used in the multivariate models when covariate data were unavailable.

For the multivariate analysis of predictors of REE, several interactions were noted. To simplify these, continuous variables were transformed into categorical ones as follows: REE was dichotomized at the median value of 1,785 kcal/day. Testosterone was divided into tertiles: upper tertile mean 21.5 pg/ml; middle tertile mean 19.2 pg/ml; lower tertile mean 14.9 pg/ml. TNF- α was divided into tertiles: upper tertile mean 6.42 ng/ml; middle tertile mean 3.39 ng/ml; lower tertile mean 1.36 ng/ml.

RESULTS

Participants. One hundred seventy-two participants contributed 190 observations, with a mean duration from start to end of the intervals of 6.5 mo. The study population was predominantly Caucasian, with a mean age of 41 yr (Table 1). The primary HIV infection risk was injection drug use in 15% and homosexual contact in 78%. Sixty-three percent of the population had met the definition of AIDS, and 43% were taking HAART during the period used for this analysis, consistent with clinical practice patterns during the period when these samples were obtained (1995–1997).

The primary goal of the study was a longitudinal analysis of change in LBM after the date of PBMC culture, with a mean follow-up time of 8.7 mo. The mean change in weight during the observation period was a loss of 0.02 kg but ranged from loss of 12.2 kg to a gain of 8 kg. The mean change in LBM was loss of 0.2 kg, but again the range of responses was wide, from loss of 9.4 kg to gain of 6.5 kg. Similarly, the mean change in REE was 6.8 kcal/day, with a range of –431 to +430 kcal/day. Weight loss of >2 kg occurred in 23% of the participants, and weight loss of >5% occurred in 11%. Weight loss of $\geq 10\%$ (AIDS wasting) occurred in

Table 1. Description of the study population

Variable*	n	Mean	SD	Range
Age, yr	190	41	8.1	23–70
Energy intake, kcal	190	2,827	759	847–4840
Protein intake, g/day	190	107	36	26.3–239
CD4 cell count, mm ³	190	346	220	0–1079
Log HIV RNA, copies/ml	175	3.7	1	2.3–5.8
Physical activity, mets/wk	187	35	4.8	30–62
Physical function, score	190	80	21	16.7–100
Demographics				
%Taking HAART	171	43		
%AIDS	190	63		
%IVDU	189	15		
%MSM	190	78		
%White	190	72		
Free testosterone, pg/ml	166	18	6.5	3.6–36.5
IL-1 β , ng/ml*	190	4.54	1.2–8.8	0.01–21.97
TNF- α , ng/ml*	190	3.74	0.8–6.3	0.9–19.70
Δ Weight, kg	190	+0.13	3.4	–12.2–+8
Δ LBM, kg	160	–0.2	2.7	–9.4–+6.5
Δ REE, kcal	132	+6.8	200	–431–498

HAART, highly active antiretroviral therapy; IVDU, intravenous drug user; MSM, men who have sex with men; LBM, lean body mass; REE, resting energy expenditure; TNF- α , tumor necrosis factor- α ; IL-1 β , interleukin-1 β ; Δ , change; n, no. of observations. *Median and interquartile range shown if variable is not normally distributed.

Table 2. Bivariate models of change in LBM and REE

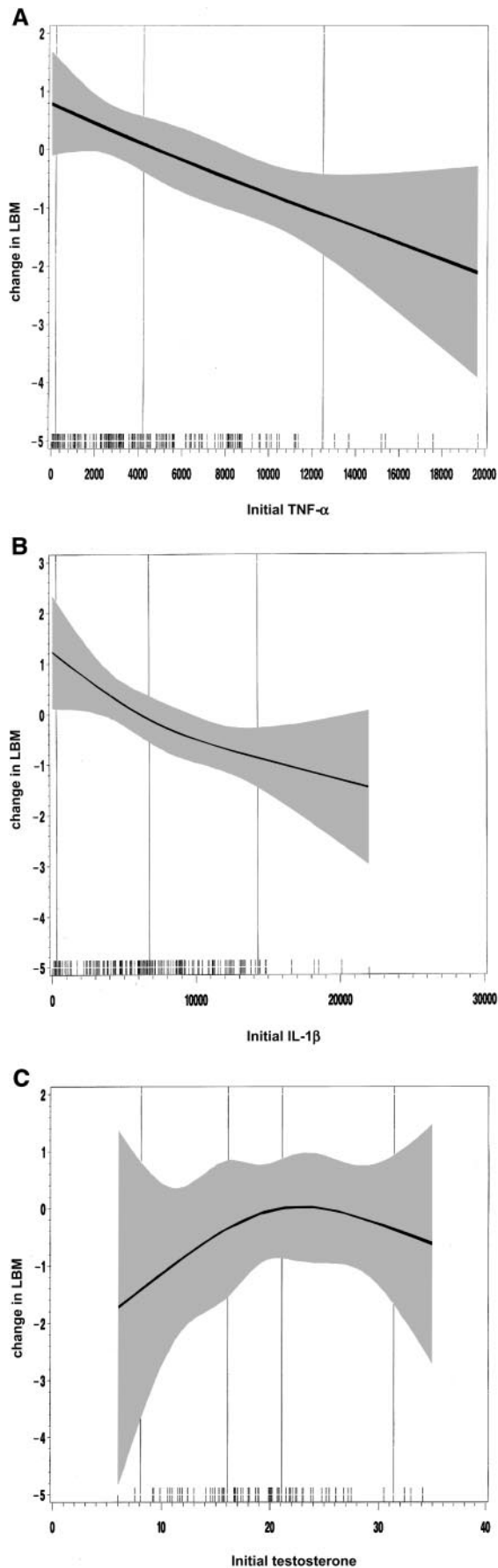
Variable	Δ LBM, g		Δ REE, kcal/day	
	β	P	β	P
Interval between visits, mo	8.7	0.4	1.0	0.06
PBMC TNF- α , ng/ml	–150	0.001	9.0	0.04
PBMC IL-1 β , ng/ml	–130	0.002	7.0	0.04
Serum free testosterone, pg/ml	41	0.3	4.5	0.10
Age, yr	–0.9	0.9	–1.5	0.45
Energy intake, kcal/day	–0.2	0.3	0.015	0.46
Protein intake, g/day	–2.7	0.7	–0.52	0.21
Physical functioning, 1 point	16.5	0.05	–1.4	0.03
Physical activity, mets/wk	3.7	0.3	–5.3	0.04
CD4 count, mm ³	2.8	0.001	–0.17	0.005
Viral load, log RNA	–217.2	0.3	26.4	0.06
HAART use (yes/no)	–24.4	0.90	–75	0.01
IVDU				
Current user	–3,060	0.002	312	0.001
Past user	637	0.3	–80	0.1
Never user	ref	ref	ref	ref
AIDS (yes/no)	–974	0.01	30	0.4
MSM (yes/no)	578	0.3	–25	0.27
Race (white/non-white)	500	0.3	19	0.6

PBMC, peripheral blood mononuclear cells; ref, reference category; β , regression coefficient.

only 2% of the observations. LBM loss of >1 kg occurred in 35% of the cohort, and LBM loss of >5% occurred in 12.2%. Among intervals where LBM loss exceeded 1 kg, the mean (\pm SD) change in weight was 1.4 \pm 3.2 kg; among intervals with LBM loss of >5%, the mean change in weight was loss of 2.9 \pm 3.3 kg. A rise in REE of >200 kcal/day ($\sim 10\%$ of median REE) was found in 17.7% of the subjects. There was no significant correlation between Δ LBM and Δ REE ($r = 0.01$, $P = 0.9$). The prevalence of hypogonadism, defined as serum free testosterone <12 pg/ml, was 17.5%.

Predictors of change in LBM. In crude models, both TNF- α [regression coefficient (β) $\beta = -150$ g LBM \cdot ng⁻¹ \cdot ml⁻¹, $P < 0.04$] and IL-1 β ($\beta = -130$ g LBM \cdot ng⁻¹ \cdot ml⁻¹, $P < 0.05$) production were inversely associated with Δ LBM (Table 2). Higher baseline CD4 count ($\beta = 280$ g LBM/100 CD4 cell increase, $P < 0.001$) and physical function score ($\beta = 165$ g LBM/10-point increase in physical function score, $P < 0.05$) were associated with gain in LBM. Active injection drug use was associated with loss of LBM ($\beta = -3,060$ g LBM compared with nonusers, $P < 0.002$), as was the diagnosis of AIDS (-974 g LBM, $P < 0.01$). There was no relationship between Δ LBM and free testosterone concentration ($\beta = 41$ g LBM \cdot pg⁻¹ \cdot ml⁻¹, $P < 0.3$), age, energy or protein intake, viral load physical activity, HAART use, or race. In addition, there were no significant associations (nonlinear or linear) between cytokines and viral load or cytokines and CD4 count.

When the relationship between Δ LBM and TNF- α was plotted using a cubic spline analysis, the relationship was linear ($P < 0.006$, Fig. 1A). IL-1 β (Fig. 1B) also predicted Δ LBM in a linear ($P < 0.003$) manner. However, testosterone did not significantly predict Δ LBM ($P = 0.3$ and 0.64 for linear and nonlinear associations, respectively; Fig. 1C). Moreover, we observed an inverse linear cross-sectional relationship



between free testosterone concentration and TNF- α production (linear association $P < 0.02$, Fig. 2A). There was also a significant inverse linear association between free testosterone and IL-1 β production (linear association, $P = 0.02$) in the spline analysis (Fig. 2B).

In multiple linear regression (Table 3), there was a significant association between Δ LBM and TNF- α ($\beta = -110$ g LBM/ng, $P < 0.02$) after adjusting for covariates that were significantly associated with Δ LBM in bivariate analysis (CD4 count, IDU status, physical functioning, and AIDS status). There was also a comparable inverse association with IL-1 β in a similar model ($\beta = -110$ g LBM \cdot ng $^{-1}\cdot$ ml $^{-1}$, $P < 0.01$, Table 3). In addition, there was an interaction between baseline percent body fat and IL-1 β , but not TNF- α , in terms of the effect of subsequent loss of LBM: men with high body fat lost more LBM for each nanogram per milliliter of IL-1 β produced by their PBMC, whereas men with low body fat were relatively protected from such loss. TNF- α and IL-1 β were not included in a single model because of collinearity problems.

Predictors of change in REE. In bivariate linear regression, change in REE was associated with TNF- α , IL-1 β , baseline viral load, CD4 count, physical activity, physical functioning, HAART use, and IDU status (Table 2). In a multivariate linear regression model that contained cytokines and testosterone ($n = 58$ observations), TNF- α ($P < 0.03$) and testosterone ($P < 0.05$) were both associated with Δ REE after adjusting for viral load, CD4 count, physical functioning, HAART use, and IDU status (Table 4). There was a trend toward an association between REE and viral load ($P < 0.06$). Significant interactions were found between TNF- α and testosterone, TNF- α and baseline REE, and also between testosterone and baseline REE in this model. IL-1 β was not a significant predictor of change in REE in multivariate linear models.

DISCUSSION

Although the incidence of classical AIDS wasting was $<2\%$ in the cohort of men reported here, over one-third of patients lost ≥ 1 kg of LBM over an average follow-up of 8 mo, and loss of $>5\%$ of LBM occurred in 12% of the men. Loss of 5% of LBM is especially important because it has previously been shown to predict a poor outcome in studies of HIV-infected adults as well as in other populations (1, 2, 13, 31). In this study, we found that 23% of the patients lost ≥ 2 kg of weight over an average follow-up of 8 mo. These changes occurred in our patients even though 43% were taking HAART. These data are consistent with our previous report from the entire Nutrition for Healthy Living cohort (30), suggesting that our subgroup was representative of the entire study cohort.

Fig. 1. Cubic spline analysis of predictors of change in lean body mass (LBM) over the follow-up period: A: baseline peripheral blood mononuclear cell (PBMC) production of tumor necrosis factor (TNF)- α ; B: PBMC production of interleukin (IL)-1 β ; C: serum free testosterone.

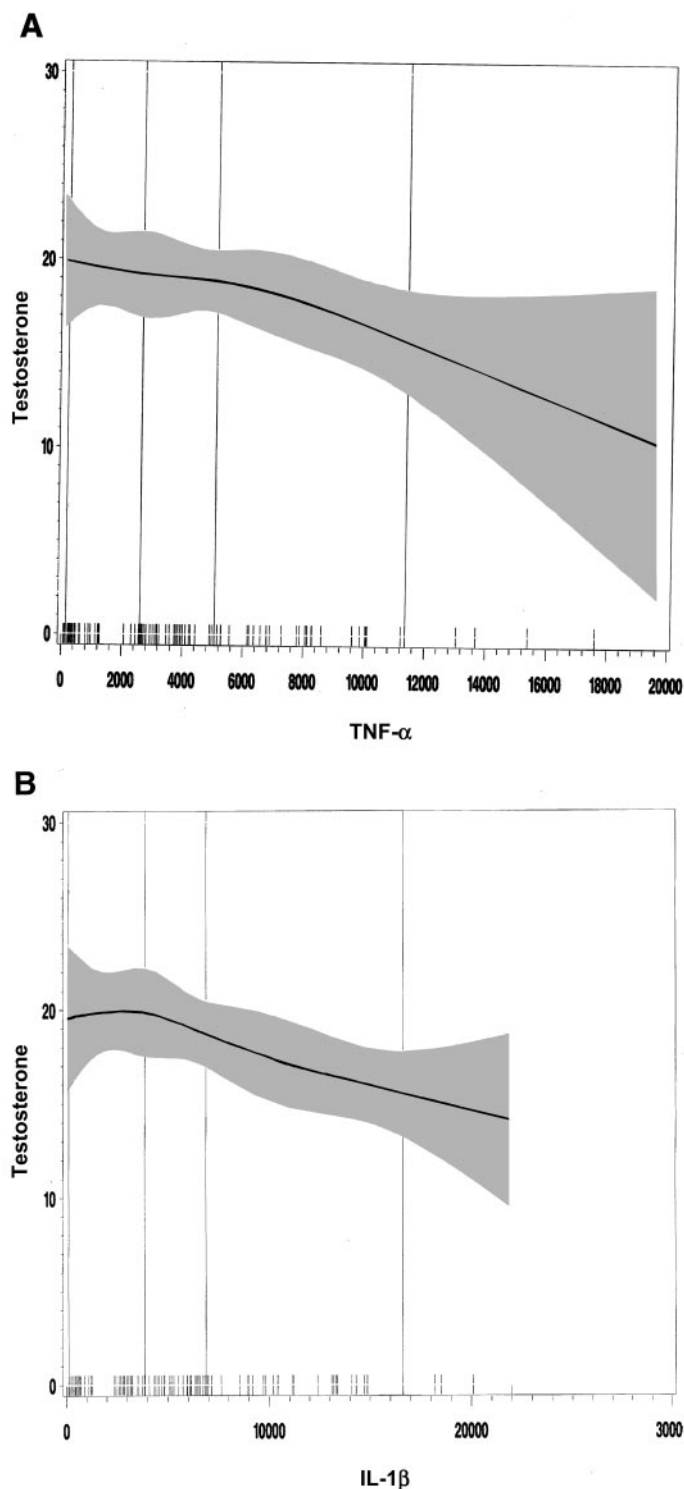


Fig. 2. Cubic spline analysis of the relationship between serum free testosterone concentration and PBMC production of TNF- α (A) and IL-1 β (B).

The results of this study indicate that loss of LBM and rise in REE, the hallmarks of cachexia as we have previously defined it (22), persist even with aggressive antiretroviral therapy. Furthermore, these data indicate that the catabolic cytokines TNF- α and IL-1 β , interacting with free testosterone, are important deter-

minants of change in LBM and REE. In contrast to previous studies indicating that hypogonadism is common among patients with classical AIDS wasting, our data demonstrate a relatively lower prevalence of hypogonadism in the era of HAART. Whereas testosterone is an independent predictor of muscle mass in hypogonadal men with AIDS wasting (10), we found no independent effect of serum free testosterone on change in LBM or REE after adjusting for other variables in our largely eugonadal population of HIV-infected men. The inverse relationship between testosterone and cytokine production suggests that, in this population, testosterone may act as a modulator of the effect of TNF- α and IL-1 β and not as a direct determinant of body composition or metabolic rate.

The relationship between PBMC production of TNF- α and IL-1 β on the one hand and change in LBM and REE on the other parallels our previous findings in another cachectic condition, rheumatoid arthritis (24), but has not been demonstrated in HIV disease in the post-HAART era before. Only TNF- α promoted hypermetabolism (elevated REE), whereas both TNF- α and IL-1 β were significantly associated with protein catabolism (loss of LBM). Although the effects are generally small, both the long duration of exposure to these cytokines in HIV infection and the potential for very high levels of production suggest that the cumulative effects of even small excesses in cytokine production over many months can be clinically significant. These associations were robust and persisted after adjusting for factors known to affect LBM, such as injection drug use, the presence of AIDS, and markers of viral load and CD4 status. Although TNF- α has been implicated in the development of lipodystrophy due to HIV infection and HAART exposure (15, 19), the present report is the first to show a link between PBMC production of TNF- α and IL-1 β , and cachexia in weight-stable HIV-infected adults.

The epidemiological nature of this study, using nearly 200 observations, limited us to measuring PBMC cytokine production rather than performing muscle biopsies to examine cytokine levels at the target tissue. However, we have shown that PBMC cytokine production is more closely linked than are serum or plasma cytokines to metabolic disturbances in another catabolic disease, rheumatoid arthritis (24); similar data are now emerging in age-related sarcopenia (R. Roubenoff, unpublished observations). Moreover, in a subset of the present study, we have found that PBMC cytokine values discriminated between wasted and unwasted HIV-infected adults better than did serum cytokines (L. W. Abad, R. Parker, and R. Roubenoff, unpublished observations). PBMC, which are primarily monocytes and lymphocytes, are directly representative of the circulating component of the immune system and in HIV disease are depleted of CD4 cells in proportion to the patient's clinical status. Thus the PBMC compartment is a reasonable one in which to obtain a valid measure of the patient's propensity to produce catabolic cytokines. Because PBMC circulate throughout the body, they are capable of delivering

Table 3. *Multivariate linear model of determinants of ΔLBM*

Variable	TNF-α Model Estimate (β)	P Value	IL-1β Model Estimate (β)	P Value
Cytokine, ng/ml	-110	0.01	-110	0.02
Physical functioning, 1 point	8.9	0.01	10	0.2
CD4 count (100 cells/ml)	1.4	0.15	1.5	0.15
AIDS status (yes/no)	-626	0.2	-522	0.3
IVDU				
Current user	-2,231	0.02	-1,910	0.07
Former user	897	0.15	818	0.2
Never user	ref	ref	ref	ref
Body fat (%) × IL-1β interaction	NA	NA		<0.0001
High body fat (=25%)			-215	
Intermediate body fat (15–24%)			ref	
Low body fat (<15%)			-55	

Values are expressed in grams. NA, not applicable.

catabolic signals to various organs, including the muscle and viscera. Furthermore, the outcomes of whole body metabolic changes, such as in REE and LBM, indicate that a whole body measure of immune function such as PBMC cytokine production be considered in preference to a localized measure in one organ.

The finding of an interaction between body fat and IL-1β production is a novel one and suggests that body fat can modulate the effect of cytokines on body composition in ways that have not been reported previously. It is not clear how fat can reduce the catabolic effect of IL-1β. Because fat cells can produce leptin, TNF-α, IL-6, and other cytokines, adipose tissue could directly modify IL-1β effects via its own cytokine secretion, which is not detected in assays of PBMC cytokine production (27). Alternatively, body fat may be a marker for genetic heterogeneity in cytokine expression or sensitivity (18) rather than a causative factor in its own right.

Interestingly, neither dietary energy nor protein intake predicted loss of LBM in this study. The mean

reported intakes were 2,827 kcal/day and 107 g protein/day, which are well within recommended intakes for this population, even without allowance for under-reporting (28). This finding is identical to the situation in rheumatoid arthritis and underlines our previous contention that cachexia, unlike wasting, is not a problem of inadequate intake but rather one of cytokine-driven altered metabolism that occurs even in the presence of adequate or even superadequate intake (22).

Several limitations of this study should be noted. First, we used BIA as our method of body composition assessment, and this method has limited precision (SEE of ~2 kg), as we have documented elsewhere (20). However, the usefulness of BIA was maximized by use of population-specific BIA equations validated against DEXA (14). Although BIA is of limited utility in the evaluation of individuals, it is strongest when applied to a large population, as was the case here. We also did not use BIA to calculate smaller body composition compartments than LBM, to avoid the larger error that occurs in such situations.

Another important limitation is that we report only on men in this study, and these men had to have adequate venous access to permit the PBMC blood draw. This is unfortunate, but we had only 39 women in our sample and thus did not have power to detect any significant associations. Furthermore, there is greater error in the measurement of the low levels of free testosterone present in women, so that the relationship between testosterone and cytokine production is more difficult to detect. We are continuing to recruit and study women to achieve an adequate sample size and hope to be able to report on them in the future. The effect of selecting men with reasonable venous access would be to reduce the prevalence of long-term injection drug users. Because those patients are likely to have more severe cachexia and poor dietary intake, it is likely that their exclusion would bias the study results toward the null rather than account for the observed findings. However, short of performing arterial punctures, it is not practical to include these patients in relatively large studies of cell culture data such as this one.

Table 4. *Multivariate linear model of determinants of ΔREE*

Variable	Estimate (β)	P Value
Interval between visits, mo	1.6	0.2
TNF, ng/ml		0.03
High REE/low testosterone	37	
High REE/high testosterone	17	
Low REE/low testosterone	7	
Low REE/high testosterone	-13	
Testosterone, 5 pg/ml		0.05
High REE/low TNF	14	
High REE/high TNF	-63	
Low REE/low TNF	55	
Low REE/high TNF	-22	
Log HIV RNA, copies/ml	47	0.06
Physical function	-1.8	0.1
CD4 count, mm ³	-0.1	0.3
HAART use (yes/no)	31	0.6
IVDU		
Current user	315	0.05
Former user	-138	0.1
Never user	ref	ref

Values are expressed in kcal/day. See PATIENTS AND METHODS for definitions of high and low variables.

In conclusion, this study shows for the first time that cachexia, separate from wasting, is a prevalent clinical problem in HIV-infected men, even in the era of HAART. In addition, the results of this study support the notion that cachexia is an immunologically driven process rather than a simple problem of inadequate intake. The continual presence of cachexia despite HAART treatment suggests that metabolic complications of HIV infection will continue in the future and that physicians should consider routine measurements of body composition even in patients whose virological status is under good control. This can be done using easily available clinical techniques such as anthropometry or bioelectrical impedance. Furthermore, specific anabolic therapy, either pharmacological or by use of exercise (5, 23), should be considered for treatment of patients who show evidence of loss of >5% of LBM, as this level of loss has been shown to increase mortality and complications in HIV-infected adults. Pharmacological therapy should be used with caution, however, because it may exacerbate insulin resistance and lipid abnormalities. In contrast, exercise is more likely to be beneficial without untoward side effects. The results of this study warn against a complacent attitude toward nutritional complications of HIV infection, even in the era of highly active antiretroviral therapy.

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